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USE OF TESTOSTERONE TO TREAT IMPAIRED GLUCOSE TOLERANCE AND INSULIN RESISTANCE AND METHOD FOR SCREENING FOR INSULIN RESISTANCE IN ADULT ONSET DIABETES AND SYNDROME X

This application claims priority of United States Provisional Application Serial No. 60/066,504, filed November 24, 1997.

Background of the Invention

I. Field of the Invention

The subject invention is directed to using pharmacologically effective amounts of hormones to prevent the manifestations and medical complications of insulin resistance and, more particularly, to the administration of testosterone and its derivatives to treat insulin resistance-based conditions.

II. Description of the Prior Art

Insulin, Insulin Resistance and Diabetes

Insulin resistance is an essential component in the malfunction of the cell. The movement of glucose from the blood stream into the cell where it is used for energy is the basis for cellular homeostasis. Recent discoveries have identified three separate components to the process termed "insulin resistance." These separate components are hyperglycemia (elevated serum levels of glucose), hyperinsulinemia (elevated serum levels of insulin), and elevated tissue levels of glycogen and glucose. When an individual has either hyperinsulinemia or elevated tissue levels of glycogen and glucose, the term "insulin resistant" is applied, because the addition of insulin does not correct these individual components.

Insulin plays an essential role in the body function. It was originally noted in diabetes mellitus because the absence of insulin often resulted in death of the individual. Banting and Best (1922) extracted insulin from the pancreas and demonstrated its usefulness in diabetic animals. Prepared in crystalline form by Abel (1926), the establishment of the amino acid sequence by Sanger (1960), the complete synthesis of the hormone by Katsoyannis (1966) and the development of various preparations with different half lives completes the historical perspective of insulin. See Randall H. Travis and George Sayers, *The Pharmacological Basis of Therapeutics,* Ch. 71 Insulin and Oral Hypoglycemic Drugs 1581-1603 (Louis S. Goodman and Alfred Gilman eds., 4th ed. 1970).

Insulin's mode of action on carbohydrate, protein, and fat metabolism remains the subject of intense investigation since its discovery seventy years ago. For diabetes is identified by hyperglycemia, elevated glucose levels in the blood. In juvenile onset diabetes, destruction of the beta cells of islets of Langerhans of the pancreas creates an insulin deficiency, which prevents glucose uptake by the cells. But in adult onset diabetes, representing 90% of all diabetes, these patients demonstrate an increase in insulin production. This represents a cellular defect so that normal levels of insulin are not effective in clearing glucose from the blood stream. The condition in which the body produces an excess of insulin is referred to as "insulin resistance."

Levine (1949) and associates made a notable contribution when they developed the concept that the cell membrane, under the influence of insulin,

regulates glucose utilization by determining the rate at which the molecule passes from extracellular to intracellular fluid, and thereby affects the rate of oxidation and the conversion to glycogen. Action of insulin at the membrane site explains many, but not all, of the varied effects of the hormone on intermediary metabolism. Current work suggests that insulin has multiple loci of action such as on the enzyme system that promotes the conversion of glucose to glycogen, and inhibits the mobilization of fat.

Travis and G. Sayers stated that "the explanation for the fact that large numbers of diabetics required much more insulin than was estimated to be secreted by the pancreas of a normal subject was sought in terms of abnormalities in the activities of the pituitary or the adrenal cortex." (Travis and Sayers, 1970).

Although insulin replacement in adult onset diabetes is the treatment prescribed, it does not prevent the development of accelerated system disease. The etiology of the characteristic defects in the cardiovascular system of the diabetic patient (e.g., retinopathy, atherosclerosis) remains unexplained. Their onset and progress do not appear to be dramatically influenced by treatment with insulin. Intense hypoglycemia can result in insulin coma, hypoglycemic convulsions and irreversible damage to the brain. (Travis and Sayers, 1970) Hyperglycemic tissue states induce cataracts, macular degeneration, obesity, and increased mortality risks from the deposits of glucose converted into lipid material within the cells. However, to date, Traverse and Sayers (1970) state that insulin is the most effective drug therapy for states of hyperglycemia.

Elevated levels of insulin are also associated with a number of abnormal states of which only one is diabetes mellitus. Since high levels of insulin block the breakdown of fat deposits and encourage the breakdown of muscle, high insulin levels are a factor in obesity and muscle wasting. Hyperinsulinemia correlates with an increased incidence of cardiovascular disease in both men and women. Hyperinsulinemia also depresses the normal production of anabolic steroids including testosterone, DHEA and growth hormone. Hyperinsulinemia also contributes to the further worsening of insulin resistance, in part, by its ability to cause increased deposits of glucose/glycogen within the cell.

In regards to the treatment of diabetes, a number of other pharmaceutical products have been shown to lower the first characteristic finding of insulin resistance, elevated serum glucose (hyperglycemia). Sulfonamide was discovered by Janbon and coworkers (1942) to induce hypoglycemia. Loubatieres (1957) discovered that the compound had no effect on the pancreatized animal; in normal animals it increased the secretion of insulin from the pancreas. Franke and Fuchs (1955) found that carbutamide and tolbutamide, antibiotic and its derivatives, lowered blood sugar levels. These are the *sulfonylureas*.

Watanabe (1918) found that another group of compounds, the *biguanides*, lowers blood glucose levels. Although diguanides (Synthalin A) and guanidine derivatives were found too toxic for therapeutic use, *phenformin* (Ungar, 1957) was found useful with an acceptable level of toxicity, so it is used extensively today.

Other compounds show hypoglycemic potential but are not used because of their toxicity in effective doses. Salicylates lower blood glucose when given in large doses. Their hypoglycemic activity, of unknown mechanism, is too weak to justify their use in diabetes.

Travis and Sayers stated that the sulfonylureas represented a most significant contribution to the treatment of the diabetic patient over forty years of age with stable and mild diabetes. These agents have the decided advantage over insulin of being effective by the oral route. They are ineffective in the unstable diabetic and in the management of any type of diabetic during acute situations of fever, trauma, or surgery. It is very doubtful, however, that the sulfonylureas reduce the incidence of diabetic complications although they have been of great benefit in the treatment of hundreds of thousands of diabetic subjects.

Sulfonyureas should be used with caution in patients with impairment of hepatic or renal function. They are not recommended for use in patients with frank hepatic or renal insufficiency because of the important role of the liver in the metabolism of sulfonylureas and of the kidney in the excretion of the drugs and their metabolites. Intolerance to alcohol reminiscent of the disulfiram reaction has occurred occasionally in patients. These drugs are effective (for lowering serum glucose) in adult onset diabetes in whom less than 20-40 units are needed to maintain control.

Diabetes of prolonged duration seems less amenable to sulfonylureas. Onset before thirty years of age, and with unstable, ketoacidosis, these patients

require insulin and attempts to control them with oral therapy are dangerous and doomed to failure. Deaths from acidosis and dehydration have occurred in patients with unstable ketotic diabetes in whom regulation was attempted with sulfonyureas.

Phenformin, in combination with insulin, may develop ketonuria even with normal blood glucose levels. The cause in unknown and the phenformin should be discontinued. Diabetics with severe renal insufficiency or congestive heart failure are not suitable candidates for oral hypoglycemic therapy. (Travis and Sayers, 1970).

Troglitazone (Warner Lambert Co.) is the first antihyperglycemic agent which acts primarily by decreasing insulin resistance. It improves sensitivity to insulin in muscle and adipose tissue and inhibits hepatic glyconeogenesis. It is not chemically related to either the sulfonylureas, the biguanides or the alpha-glucosidase inhibitors.

Troglitazone is a thiazolidinedione anti-diabetic agent that lowers blood glucose by improving target cell response to insulin. It has a unique mechanism of action that is dependent of the presence of insulin for activity. Troglitazone decreases hepatic glucose output and increases insulin-dependent glucose disposal in skeletal muscle. Its mechanism of action is thought to involve binding to nuclear receptors (PPAR) that regulate the transcription of a number of lipid responsive genes critical for the control of glucose and lipid metabolism. Unlike sulfonylureas, troglitazone is not an insulin secretagogue.

In animal models of diabetes, troglitazone reduces the hyperglycemia, hyperinsulinemia, and hypertriglyceridemia characteristic of insulin-resistant states such as type II (adult onset) diabetes. Plasma lactate and ketone body formation are also decreased. The metabolic changes produced by troglitazone result from the increased responsiveness of insulin-dependent tissues and are observed in numerous animal models of insulin resistance. Treatment with troglitazone did not affect pancreatic weight, islet number or glucagon content, but did increase regranulation of the pancreatic beta cells in rodent models of insulin resistance.

Since troglitazone enhances the effects of circulating insulin (by decreasing insulin resistance). It does not lower blood glucose in animal models that lack endogenous insulin.

Since medical experience has demonstrated that the reduction of serum glucose in type II (adult onset) diabetes corrects ketoacidosis but does not prevent the other aspects of the disease (Travis and Sayers, 1970), effective treatment for diabetes relies on correction of both hyperinsulinemia and increased concentration of cellular glycogen. However, troglitazone, the only product recognized to lower insulin levels and the levels of cellular glycogen, has a number of potent and life-threatening side-effects. As reported to date, the side-effects include abnormal liver function tests, jaundice, drop in white blood cell count and drop in hemoglobin which may persist for two years. It is recommended not to be used

in combination with oral contraceptives, terfenadine, and cholestyramine, because it lowers the effective blood level of these drugs by up to 30 percent.

In animal studies, troglitazone was administered daily for 104 weeks to male and female rats in various doses. In female rats, there was a statistically significant increase in sarcomatous tumors at the high (200 mg/kg) dose (47-fold greater than estimated human exposure of parent compound). However, these findings are of unknown clinical relevance as this dose was associated with excessive mortality and is considered to have surpassed the maximum tolerance dose. (Physicians' Desk Reference 1998)

Because of this, the PDR notes that troglitazone is only indicated for use with type II diabetes currently on insulin therapy whose hyperglycemia is inadequately controlled (HbA_{1c} >8.5%) despite insulin therapy of over 30 units per day given as multiple injections.

But impaired glucose tolerance, hyperglycemia and insulin resistance are now seen as etiologic elements to much more than adult onset diabetes mellitus.

Reaven (1988) defined Syndrome X as the constellation of various components: insulin resistance, glucose intolerance, hyperinsulinemia, increased VLDL triglyceride, decreased HDL cholesterol, and hypertension. Kaplan (1989) noted the coexistence of upper body (central) obesity, glucose intolerance, hypertriglyceridemia, and hypertension. Black (1996) added coronary artery heart disease to this syndrome. Therefore, by the consensus of these and other researchers, the constellation of laboratory findings of insulin resistance is the

underlying or consistent component of diabetes, obesity, hypertension, dyslipidemia and heart disease. Insulin resistance is the key element of Syndrome X. Syndrome X affects 70-80 million people, almost one-third of Americans, is a linked to obesity and weight gain, is associated with diabetes, is associated with high blood pressure, is a common factor in cardiovascular diseases and stroke, and is a primary cause of lowered metabolism and fatigue.

However, although many hundreds of articles have attempted to elucidate a unifying biochemical marker for this disorder, none has been documented. Rather than searching for a genetic defect, Applicant sought to find a consistent change in hormonal parameters in these individual disease states a pharmacologic treatment for such.

That entity has been found to be (1) the unbound testosterone fractions as calculated by the Testosterone Ratio Tests, (2) pharmacologic doses of testosterone and/or its derivates, and (3) application in both men and women. Applicant sought to discover why many of these diseases were linked to hormonal changes as well as to insulin resistance. Applicant then sought to discover the pharmacologic range of stated hormone that affected changes in insulin resistance and reversal of laboratory and clinical findings of these diseases.

For descriptive purposes, all medical conditions with insulin resistance, including adult onset diabetes, will be included under the designation of Syndrome X. For the purpose of defining the measured laboratory components of insulin resistance, references are made to (1) hyperglycemia, (2) hyperinsulinemia and (3)

increased tissue glycogen and their respective laboratory tests (1) fasting serum glucose, (2) fasting serum insulin and (3) serum elevated hemoglobin A_{1c} (HbA_{1c}).

Because normal insulin metabolism is necessary for good health and to optimize delivery of many nutrients, interest in this area continues to increase within both the medical and pharmaceutical communities. Doctors and scientists all over the world are starting to see that many seemingly unrelated diseases are in fact linked to a malfunction in insulin and/or blood-sugar metabolism. Insulin's primary role is to lower blood-sugar levels by transporting carbohydrate energy (glucose) into and out of muscle and liver cells.

Secondarily, insulin helps pull amino acids into the cells, turns on protein synthesis and promotes fatty acid/triglyceride storage. Problems with the body's ability to regulate blood glucose crop up if the cells don't readily accept blood sugar or if insulin doesn't properly bind to its glucose transport receptors. When normal amounts of insulin do not reduce blood sugar after meals, the body secretes more and more insulin until serum glucose levels fall, sometimes too far. For example, the urge to snack at around 4 p.m. and 9 p.m. is typically tied to wild fluctuations in blood sugar levels. This is not to be confused with symptoms of either a potential diseased state or full-blown insulin resistance.

Insulin resistance most likely begins as a genetic predisposition that becomes manifested by the over-consumption of simple and refined carbohydrates, the lack of adequate nutrients combined with being sedentary (Grimditch, 1988). Consuming too many processed simple carbohydrates coupled

with inadequate nutrient intake are common shortcomings of the typical American diet, and, of course, the American lack of adequate exercise. Conversely, and not surprisingly, diets and nutrients that reduce the amount of insulin required by the body also reduce the tendency toward excessive production of insulin. (Murray, 1991) Exercise has also been shown to be an excellent modality for improving insulin sensitivity.

Well-known for its ability to store glucose in muscle and increase protein synthesis and muscle mass, insulin has only recently been recognized as the primary anabolic hormone produced in the body. Some researchers believe insulin is in fact more important to lean muscle tissue than the better-known anabolic hormones testosterone and growth hormone. Unfortunately, insulin resistant individuals also suffer from a drop in testosterone and growth hormone. Out-of-control insulin metabolism tends to make you gain body fat and catabolize muscle. With increased insulin resistance both the number of calories stored as fat and the amount of fat produced by the liver from carbohydrates gets worse.

Because of the impaired ability of muscle to release glucose from the muscle, these individuals have fatigue and a decreased ability to exercise. The problem of insulin resistance potentiates and accelerates the development of the various disease states of Syndrome X.

Proper insulin function is of paramount importance to those dealing with the constellation of symptoms associated with improperly functioning insulin metabolism. In terms of nutrition, over consuming any of the macro nutrients (and

particularly simple carbohydrates) should be avoided. As for supplements, micro-nutrients such as vitamins C and E, magnesium, omega-3 fatty acids, chromium and vanadyl sulfate have been shown to improve insulin sensitivity. (Murray, 1991) Diabetics, people with Syndrome X, and athletes who want to improve their insulin metabolism/sensitivity might choose to include these nutrients in their diets. And of course, regular exercise improves insulin sensitivity.

For all individuals, understanding insulin metabolism and the damage caused by too much glucose and insulin is essential for good health. At every age, one wants to be gaining muscle, losing body fat and using diet and those therapies that will prevent the spiral into insulin resistance. Avoiding Syndrome X and the many diseases associated with it means regular exercise, adequate and correct nutrient intake, shunning health-degrading substances and using some common sense. For physicians and health professionals, closer attention to prevention and early diagnosis will simplify treatment and minimize the manifestation of diseases associated with Syndrome X.

Although various dosages of testosterone have been used in the treatment of medical conditions, and there are at least three observational references attesting to the positive effects of testosterone on diabetes (Moller and Einfeldt 1984, Carruthers 1996, Mauriello et al. 1997), there exists no previous documentation in either adult diabetes mellitus or any other disease associated with Syndrome X of the methodology and effects of using these three claimed components to effect treatment: (1) pre-selection of patients based on the

testosterone/derivatives ratio testing, (2) testosterone dosing schedule, based on reaching specific serum concentration of testosterone and/or its derivates and (3) documentation of the reversal of all three components of insulin resistance: hyperglycemia, hyperinsulinemia, and abnormal glycosylated hemoglobin A_{1c} levels with testosterone and/or its derivatives.

Summary of the Invention

According to the present invention, there is disclosed a method for diagnosing, treating and evaluating those suffering with insulin resistance. This is accomplished in an individual by calculating a ratio of unbound testosterone and/or its derivative, administering a pharmacologically effective, serum-glucose, serum insulin, and glycosylated hemoglobin lowering amount of testosterone or the analogs, derivatives, and pharmaceutically acceptable salts, esters, amides, and prodrugs thereof to the subject. Furthermore, there is also disclosed a method for performing a testosterone test which includes the steps of obtaining a serum sample, assaying the serum sample to determine both the concentration of total testosterone and sex hormone binding globulin (SHBG) in the sample, and calculating the ratio of the concentration of total testosterone and/or its derivatives to the concentration of SHBG in the sample in order to ascertain predictive and diagnostic information therefrom as to the state of insulin resistance. Also disclosed is a method for follow-up in determining the effects of an anti-hyperglycemia treatment on a subject which includes the steps of obtaining a serum sample from the subject, assaying the serum sample to determine both the

concentration of total testosterone and the concentration of SHBG in the sample, and calculating the ratio of the concentration of total testosterone to the concentration of SHBG in the sample.

There is also disclosed a dosage schedule for inducing hypoglycemia in a subject having hyperglycemia including the steps of administering a pharmacologically effective serum-glucose lowering amount of testosterone or the analogs, derivatives and pharmaceutically acceptable salts, esters, amides, and prodrugs thereof to the subject.

Brief Description of the Drawings

The following detailed description is best understood with reference to the following drawings in which:

Figure 1 is a graph illustrating serum glucose levels versus administration of testosterone over time and amount on insulin administered versus testosterone injection over time in a woman;

Figure 2 is a graph illustrating serum glucose levels versus testosterone injection over time and Micronase (sulfylurea/glyburide) levels versus testosterone injections over time in a man;

Figure 3 is a graph illustrating serum glucose levels versus testosterone administration over time and insulin use versus testosterone administration over time in an insulin resistant man;

Figure 4 is a graph illustrating glycosylated hemoglobin (HbA_{1c}) versus testosterone administration in man over time; and

Figure 5 is a graph illustrating the differential effect of sex hormone binding globulin (SHBG) on the percentage of tracer treated testosterone and estradiol.

Detailed Description of the Invention

Testosterone Drug: Administration in pharmacological doses

Both insulin and oral hypoglycemic agents derive their hypoglycemic effects by increasing utilization of exogenous or endogenous insulin. The present invention provides for the administration of pharmacological doses of testosterone, analogs, derivatives and pharmaceutically acceptable salts, esters, amides, and prodrugs thereof, with the intention to lower tissue glucose/glycogen levels to correct the physiological basis of insulin resistance. Thereafter, both men and women patients experience the lowering or blood glucose, hyperglycemia, and the lowering of insulin levels, hyperinsulinemia. Both clinically and under biological test conditions, both men and women confirm a lowering of insulin resistance as noted with a decrease in HgB A1C.

This can be accomplished by the administration of testosterone and its many derivatives including testosterone enanthate, testosterone proprionate, testosterone cyprionate, nandrolone phenproprionate and decanoate, methyl-testosterone, fluoxymesterone, oxymetholone, methandrostenolone methandriol, norethandrolone, stanozol, dihydrotestosterone and derivatives and/or combinations thereof. These preparations are usually given by intramuscular or subcutaneous pellet placement. Transdermal gels of specific formulations may

result also in comparable serum levels and results. Excluded from usage are usually the oral preparations of testosterone, such as methyl testosterone, because they rapidly cause liver toxicity. Rectal suppositories of testosterone may or may not be useful although they have the potential to mimic the side-effects of oral medication.

Testosterone has no interaction with sulfonylureas, biguanides and insulin making it suitable for combination therapy without concern of drug interaction. In clinical application, testosterone can be added and in time, the other agents discontinued or reduced.

Testosterone at the present time is used to treat hypogonadism, the absence or lack of testosterone. Medical references in Goodman and Gilman, 1970 (see generally) are made to the use of testosterone in the treatment of the following conditions: osteoporosis, menstrual disorders, wasting states, and refractory anemia (aplastic anemias, hemolytic anemias and the anemias associated with lymphoma, leukemia and various other disorders). Medical literature also reports scattered treatments of diabetes, rheumatoid arthritis, cluster headaches, impotence in men and depression.

The present invention also provides for the utilization of the testosterone ratio tests, TRTs, which comprises obtaining a serum sample from the subject or patient and assaying the serum sample to determine both the concentration of total testosterone and its primary derivative, dihydrotestosterone, present in the sample, and the concentration of sex hormone binding globulin (SHBG) in the sample.

The amounts of total testosterone and SHBG are used to calculate a ratio of the concentration of total testosterone to the concentration of SHBG in the sample. Likewise, the calculation is made for dihydrotestosterone and SHBG. These ratios correlate more accurately to the present condition of the individual than the free or "unbound" testosterone level measured directly.

Applicant has found that this ratio is predictive for screening or identifying subjects of patients who may have or be predisposed to insulin resistance. This includes men and women with adult onset diabetes, cardiovascular disease, glucose intolerance, central obesity, and other diseases or conditions associated with Syndrome X. Data confirming this correlation in women with heart disease is shown below in Example 6.

Additionally, the testosterone ratio test can be used once testosterone treatment has begun to monitor the most effective level of treatment. In those individuals with low testosterone ratio tests, those with higher SHBG need additional testosterone therapy to deliver a subsequent fall in the laboratory findings of insulin resistance (hyperglycemia, hyperinsulinemia, and elevated glycosylated hemoglobin). While the normal male's TRT ranges from approximately 0.6 to 1.2, an ideal testosterone ratio ranges from 5.0 to 12.0 in those individuals undergoing treatment with parenteral testosterone for insulin resistance.

Dosages of testosterone and/or its derivatives can be given in such a manner to maintain serum levels at twice that considered maximum range for an

adult male. For example, if a standard reference laboratory has a normal range of serum testosterone of 50-1200 mg/dl, then by the present methodology, for both men and women the therapeutic range of testosterone dosing would be reached with either frequent injections of testosterone and/or its derivatives or by placing testosterone pellets in the patient until the serum level approximated 2000-2800 mg/dl. However, modification of the therapeutic target level of testosterone is noted in reference in the previous paragraph.

Those skilled in the art are easily able to identify patients having hypoglycemia, diabetes, insulin resistance and Syndrome X and its related components. For example, those skilled in the art would recognize and diagnose a subject having central obesity, hypertension, low HDL cholesterol, etc.

A therapeutically effective amount is an amount of testosterone or analogs or derivatives thereof, that when administered to a patient, ameliorates a symptom of the disease.

The testosterone, analogs, or derivatives thereof of the present invention can be administered to a patient either alone, with other hypoglycemic agents or as part of a pharmaceutical composition. The compositions can be administered to patients parenterally (intravenously, intramuscularly, subcutaneously or transdermally).

Compositions suitable for parenteral injection may comprise physiologically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, and sterile powders for reconstitution into sterile

injectable solutions or dispersions. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (propyleneglycol, polyethyleneglycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants.

These compositions may also contain adjuvants such as preserving, wetting, emulsifying, and dispensing agents. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, for example sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

If a suitable form of oral testosterone becomes available for treatment of these conditions of insulin resistance, then solid dosage forms for oral administration include pellets, capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is admixed with at least one inert customary excipient (or carrier) such as sodium citrate or dicalcium phosphate or (a) fillers or extenders, as for example, starches, lactose, sucrose, glucose, mannitol, and silicic acid, (b) binders, as for example, carboxymethylcellulose,

alignates, gelatin, polyvinylpyrrolidone, sucrose, and acacia, (c) humectants, as for example, glycerol, (d) disintegrating agents, as for example, agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain complex silicates, and sodium carbonate, (e) solution retarders, as for example paraffin, (f) absorption accelerators, as for example, quaternary ammonium compounds, (g) wetting agents, as for example, cetyl alcohol, and glycerol monostearate, (h) adsorbents, as for example, kaolin and bentonite, and (i) lubricants, as for example, talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, or mixtures thereof. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethyleneglycols, and the like.

Solid dosage forms such as pellets, tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells, such as timed release coatings and others well known in the art. They may contain opacifying agents, and can also be of such composition that they release the active compound or compounds in a certain part of the intestinal tract in a delayed manner. Examples of embedding compositions which can be used are polymeric substances and waxes. The active compounds can also be in micro-encapsulated form, if appropriate, with one or more of the above-mentioned excipients.

Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Compositions for rectal administrations are preferably suppositories which can be prepared by mixing the compounds of the present invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethyleneglycol or a suppository wax, which are solid at ordinary temperatures but liquid at body temperature and therefore, melt in the rectum or vaginal cavity and release the active component.

Dosage forms for topical administration of a compound of this invention include ointments, powders, sprays, and inhalants. The active component is admixed under sterile conditions with a physiologically acceptable carrier and any preservatives, buffers, or propellants as may be required.

The term "pharmaceutically acceptable salts, esters, amides, and prodrugs" as used herein refers to those carboxylate salts, amino acid addition salts, esters, amides, and prodrugs of the compounds of the present invention which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of patients without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention. The term "salts" refers to the relatively non-toxic, inorganic and organic acid addition salts of compounds of the present invention. These salts can

be prepared *in situ* during the final isolation and purification of the compounds or by separately reacting the purified compound in its free base form with a suitable organic or inorganic acid and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, nitrate, acetate, oxalate, valerate, oleate, palmitate, stearate, laurate, borate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate mesylate, glucoheptonate, lactobionate and laurylsulphonate salts, and the like. These may include cations based on the alkali and alkaline earth metals, such as sodium, lithium, potassium, calcium, magnesium, and the like, as well as non-toxic ammonium, quaternary ammonium and amine cations including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. (See, for example, S.M. Berge (1977).

Examples of pharmaceutically acceptable, non-toxic esters of the compounds of this invention include C_1-C_6 alkyl esters wherein the alkyl group is a straight or branched chain. Acceptable esters also include C_5-C_7 cycloalkyl esters as well as arylalkyl esters such as, but not limited to benzyl. C_1-C_4 alkyl esters are preferred. Esters of the compounds of the present invention may be prepared according to conventional methods.

Examples of pharmaceutically acceptable, non-toxic amides of the compounds of this invention include amides derived from ammonia, primary C_1-C_6 alkyl amines and secondary C_1-C_6 dialkyl amines wherein the alkyl groups are

straight or branched chain. In the case of secondary amines the amine may also be in the form of a 5- or 6-membered heterocycle containing one nitrogen atom.

Amides derived from ammonia, C_1-C_3 alkyl primary amines, and C_1-C_2 dialkyl secondary amines are preferred. Amides of the compounds of the invention may be prepared according to conventional methods.

The term "prodrug" refers to compounds that are rapidly transformed *in vivo* to yield the parent compound of the above formulae, for example, by hydrolysis in blood. A thorough discussion is provided in T. Higuchi and V. Stella (1987) and also see generally in *Bioreversible Carriers in Drug Design*, (1987) ed. Edward B. Roche.

In addition, the compounds of the present invention can exist in unsolvated as well as solvated forms with pharmaceutically acceptable solvents such as water, ethanol, and the like. In general, the solvated forms are considered equivalent to the unsolvated forms for the purposes of the present invention.

The compounds of the present invention can be administered to a patient at dosage levels in the range of about 400 to about 1200 mg of parenteral testosterone and/or derivatives thereof per month. For a normal human adult having a body weight of about 70 kilograms, a dosage in the range of about 15 to about 40 mg of testosterone and/or derivatives thereof per kilogram of body weight per day is preferable. The specific dosage used, however, can vary. For example, the dosage can depend on a number of factors including the requirements of the patient, the severity of the condition being treated, and the pharmacological

activity of the compound being used. For example, transdermal dosage is 50-100% higher due to absorption differences from transdermal to intramuscular or subcutaneous placement. With the addition of the testosterone ratio tests, the determination of optimum dosages for a particular patient is well known to those skilled in the art. In addition, it is intended that the present invention cover compounds made either using standard organic synthetic techniques, including combinatorial chemistry or by biological methods, such as through metabolism.

The examples presented below are intended to illustrate particular embodiments of the invention and are not intended to limit the scope of the specification, including the claims, in any way.

The following abbreviations are used throughout this application:

ApoB	=	Apoprotein-(B)
BMI	=	body mass index
CAD	=	coronary artery disease
ASHD	=	atherosclerotic heart disease
F	=	cortisol
DHEAS	=	Dehydroepiandrosterone sulfate
DPC	=	Diagnostic Products Corp
DSL	=	Diagnostic Systems Lab
FT	=	free testosterone
TRT	=	Testosterone Ratio Test
HDL/Chol	=	HDL/ Cholesterol ratio
HDL	=	high density lipoprotein cholesterol
LDL	=	low density lipoprotein cholesterol
MDD	=	minimal detectable dose
Tri	=	triglycerides
RAI	=	radioimmunoassay
SHBG	=	sex-hormone-binding globulin
TC	=	total cholesterol
TRT	=	Testosterone Ratio Test
TT	=	total testosterone
WHR	=	waist-to-hip ratio

Examples

Example 1

C.W. was a 65 year old white male with a fifteen year history of insulin dependent diabetes mellitus. He was hospitalized with gangrene of the middle finger of his right hand and was scheduled for amputation. He had already had bilateral femoral-popliteal bypass surgery for vascular insufficiency of the lower extremities. Due to anorexia, he was given an intramuscular injection of 200 mg of aqueous testosterone. His fasting glucose fell from 212 to 159. Repeated dosing every other day for one week resulted in a normalization of his serum glucose and a decrease in his insulin requirement from 44 units of Humulin (insulin) (Lilly) daily to 24 units. His finger healed completely in two months making amputation unnecessary.

Example 2

A.W. was a 74 year old black female who has been an insulin dependent diabetic for fifteen years. She had two toes removed previously for gangrene on the left foot; the remaining toes showed dry gangrene and a draining ulceration. She was scheduled for amputation. She used 24 units of Humulin insulin in the morning and 16 units at night. Her fasting blood glucose was 334. Treated with 150 mg of depo-testosterone enanthate intramuscularly, three times weekly, she became euglycemic on less than 12 units daily, see Figure 1. However, due to the infected heal ulcer, amputation proceeded.

Example 3

J.G. was a 81 year old white male on oral hypoglycemic agents for ten years. Present dosage was 20 mg/day, Micronase 7 (Upjohn). He presented with ulcerations in four areas of his left foot, no dorsal pedis pulsation, and was scheduled for amputation but refused. Fasting glucose was 244. Treatment with 250 mg depo-testosterone enthanate intramuscularly three-times-weekly for four weeks resulted in J.G. becoming euglycemic and discontinuing his oral agents, see Figure 2.

Example 4

G.S. was a 43 year old white male with uncontrolled diabetes for ten years. Seen intermittently for ulceration of the feet, he presented with a serum glucose of 550 while on 100 units of Humulin. He refused admission to the hospital. Over the course of six months, he received 250 to 900 mg of aqueous testosterone subcutaneous pellets every six (6) weeks. His insulin requirements were reduced to 35 units while his serum glucose remained stable at 140 to 180 mg/dl.

The medical basis in the four cases illustrated in Examples 1-4 for the normalization of serum glucose in both insulin and non-insulin dependent adult-onset diabetes is thought to be that testosterone changes insulin resistance at the cellular level. By reversing tissue resistance to the movement of glucose into the muscle and liver cell, testosterone increases the tissue's sensitivity to insulin. The result is that the level of glucose in the serum (blood) decreases and the amount of insulin needed to effect this drop in serum glucose in reduced. Future

evaluation of cellular biochemistry under conditions of supraphysiologic levels of testosterone may show that the conversion of glycogen into glucose is also improved. This may explain why these individuals reported no problems with hypoglycemia at night. Future studies may also confirm the increase in local tissue growth hormone factors. In the last three patients, the amount of the insulin-like growth factor-1 increased significantly with testosterone treatment.

The mode of action of testosterone in these examples may be many-fold. Testosterone utilizes intracellular glycogen. Previously reported tissue biopsies confirm that the diabetic individual has elevated glycogen stores within the muscle cell. No therapy has changed this finding. However, a previous study showed that testosterone significantly lowered the amount of glycogen within the muscle cell. Therefore, testosterone increased cellular utilization of intracellular glycogen. Testosterone by its action on sex hormone binding globulin lowers insulin resistance. Previous literature confirms that insulin does not change sex hormone binding globulin but change in sex hormone binding globulin changes insulin release. By way of the above examples, the utilization of various forms of testosterone resulted in a drop in serum glucose. Thus, the amount of insulin needed to stabilize a given glucose load will be reduced. Therefore, testosterone lowers serum blood levels and lowers insulin resistance. Testosterone, by the actions of utilization of intracellular glycogen and its action on sex hormones binding globulin, allows the liver cells to reduce the storage of glycogen from the excess blood glucose. Therefore, testosterone should lower fructosamine levels.

By fact that the liver cells are able to stop storing excess glucose as intracellular glycogen, could raise liver cells are able to produce additional IGF-1. Therefore, testosterone, by clearing intracellular glycogen should raise liver production of IGF-1.

The utilization of injectable and subcutaneous pellets of testosterone lowers the sex hormone binding globulin. However, depending on the diabetic state, there will be a physiologic limit to the decrease in sex hormone binding globulin. Once this maximum individual effect is realized, higher physiologic levels of testosterone offer no additional benefit. Subcutaneous and intramuscular androgens have similar effects on sex hormone binding globulin and, therefore, insulin resistance in tissue. However, oral androgens, testosterone creams, gels and transdermal delivery systems do not have this same positive effect on insulin resistance and/or sex hormone binding globulin. Testosterone increases diabetic male erectile performance. This may be related to an increase in total testosterone, free or unbound testosterone, an improvement in circulation or a decrease in sex hormone binding globulin.

Example 5

Testosterone enanthate 300 mg was given to a subject intramuscularly twice weekly for four weeks, then continued with therapy of 300 mg every two weeks. The subject's hemoglobin A1C dropped from 9.9 in May, 1998 to 7.2 in August, 1998 to 5.5 in October, 1998 demonstrating a drop in tissue glucose stores as shown in Figure 4.

Example 6

Background:

Many studies have sought to discover a common hormonal denominator to explain the predominance of coronary arterial disease in men. There is agreement among many authors that the presence of hypo-testosteronemia (Phillips et al., 1994) (low testosterone), hypoadrenalism (Nafziger et al., 1991; Mitchell et al., 1994; and Newcomer et al., 1994) (low DHEAS) and/or increased sex hormone binding globulin (Phillips et al., 1994) (SHBG) defines a state for men for increased risk of coronary artery disease (CAD) and myocardial infarction (MI). However, there is no such consensus for women. This study was undertaken to determine if a similar association could be determined for postmenopausal women since previous researchers are in disagreement (Phillips et al. (1994); Haffner et al., 1995; Hauner et al., 1994; Barrett-Connor and Goodman-Gruen, 1995; and Cauley et al., 1990). A subset of 161 postmenopausal women were identified from the 461 women in the A.L.A.R.M. (Association for Lipids and Atherosclerosis Research in Michigan, 1995-1996) database. Both the forty-nine using estrogen supplements and the remaining 112 not using estrogen supplements had samples of their serum analyzed for estradiol (E2), total testosterone (TT) and free testosterone (FT), dehydro-epiandrosterone sulfate (DHEAS), androstenedione (A), cortisol (F), insulin and Sex Hormone Binding Globulin (SHBG).

Methods

Patients

One hundred and sixty-one postmenopausal women were identified from the A.L.A.R.M. database. Included for each was a comprehensive cardiovascular risk database and anthropologic measurements of blood pressure, body mass index and waist-to-hip ratios. Serum lipid measurements were processed under the direction of JM at the C.D.C. approved laboratory in Michigan, while maintaining frozen serum in storage at -80° Centigrade. A 2cc aliquot from this frozen serum was removed for duplicate, batch hormone analysis at the Providence Hospital research laboratory. RAI assays (see Table I) were performed for estradiol, total testosterone, free testosterone, DHEAS, androstenedione, sex-hormone binding globulin (SHBG), cortisol and insulin. Estradiol, the most potent estrogen, was measured because it both correlates with estrone in postmenopausal women (Barrett-Connor and Goodman-Gruen, 1995) and because its has relatively stronger affinity for SHBG. The method for measuring estradiol has been described previously (Cauley et al., 1990).

TABLE I

LABORATORY ASSAYS COEFFICIENTS

LAB TEST	SOURCE	MDD	Intra-assay	Inter- assay
	Coated Tube IRA			
Testosterone Total	Diagnostic (DPC) Product Corp	2.1 *ug*/dl	C.V.= 6.4%	C.V. = 9.5%
Testosterone Free	DPC	0.18 pg/ml	C.V.= 7.3%	Single assay

DHEA-SO_4	DPC	2.1 *ug*/dl	C.V.= 6.4%	C.V.= 9.5%
Cortisol	DPC	0.2 *ug/dl*	C.V.= 4.9%	Single assay
	Double Antibody Technique			
Insulin	Diagnostic Systems Lab (DSL)	1.3 mIU/ml	C.V.= 4.6%	Single assay
Estradiol	DSL	1.4 pg/ml	C.V.= 7.3%	C.V.= 1.3%
Androstenedione	DSL	0.02 *ng*/ml	C.V.= 4.5%	Single assay
Sex Hormone Binding globulin	Coated Tube immunoradio-metric assay	3 *nmol*/L	C.V.= 7/8%	Single assay

Procedures

Informed consent was obtained at the beginning of the examination after which the operator included measurements of height and weight. Anthropologic measurements were performed in triplicate for future determination of body mass index and waist-to-hip ratios. Blood pressure readings were taken in triplicate and averaged using a sphygmomanometer to the nearest digit on the right arm of the seated participant after at least a 5-minute rest period. Diabetes was defined as having a previous history of being treated with insulin or a hypoglycemic medication. Heart disease was based on the physician's record of angina associated with changes in EKG or hospitalization/ heart catheterization studies. Smoking was also considered as a co-variable in the statistical analysis.

Serum cholesterol and HDL cholesterol were determined by enzymatic procedure at the C.D.C. approved laboratory in Michigan. Insulin was measured by double extraction technique. However, although the patient's last meal was reported as the day before the evaluation, the time of day at which the blood was

drawn varied from 8:00 AM to 4:30 PM. It is unlikely that the time of assay led to any systematic bias in the association between sex hormones and cardiovascular risk factors.

Statistical Analysis

All statistical analyses were performed with SPSS version 6.1 on an IBM 200mHz personal computer. In all analyzes, a two-tailed value of P<= .05 was considered significant. In the multiple-regression model used to determine the relationship of sex hormones and risk factors for cardiovascular disease, the risk factors for cardiovascular disease were the dependent variables and the hormonal parameters the independent variables.

Even though essentially all of the estrogen (Grodin et al., 1973) and much of the testosterone (Horton and Tail, 1966) in women are derived from androstenedione, all non-testosterone hormonal parameters (estradiol, DHEAS, androstenedione, and cortisol) were considered unbound for analysis. Their ratios to sex-hormone binding globulin were also included in the analysis. To determine whether significant correlation existed between any two independent variables in the study, partial correlation coefficients were calculated after controlling for age and BMI.

Results:

The mean ± SEMs for the variables measured in the total group of 161 women and the subgroup with estrogen (49) and without estrogen supplementation (112) are shown in Table II.

The correlation coefficients for the most significant factors, BMI, WHR, and Testosterone Ratio Test (total testosterone/ SHBG) appear in Table III.

The correlation coefficients for the Testosterone Ratio Test (TRT) for 1) the total group, 2) the subset with estrogen, and 3) the subset not taking estrogen appear in Table IV.

Independent hormonal correlation coefficients were as follows:

Testosterone was significantly positively correlated with DHEAS (r=0.516, p< .000) and free testosterone (r=.945, p<.000). The assay of free testosterone was significantly and positively correlated with Androstenedione (r=.416, p<006).

Estradiol was significantly and inversely correlated with BMI (r=-.324, p<034) and WHR (r=-.313, p<.038). Estradiol divided by Testosterone ratio was significantly and inversely correlated with Androstenedione (r=-.429, p<.014), BMI (r=-387, p<.028), DHEAS (r=-.367, p<.035). Cortisol correlated significantly and directly with Androstenedione (r=.407, p<.000), and HDL (r=.199, p<018) and inversely with BMI (r=-.265, p<.001). Androstenedione correlated significantly and directly with DHEAS, Testosterone, and Free testosterone.

Note that Apoprotein-B did not correlate with any risk factors except the lipid profiles: HDL/Cholesterol ratio (r=.702, p<000), HDL (r=-.380, p<.011), LDL (r=.831, p<.000), total cholesterol (.805, p<.000), and triglycerides (r=.663, p<.000). The HDL/Cholesterol ratio correlated inversely with BMI (r=-.252,

p<.002), WHR (r=-.299, p<.000) all lipid parameters (p<.000) and DHEAS (r=-282, p<.022). The only direct correlation was to HDL correlation was positive (r=.757, p<.000).

Table II shows the means and standard deviations for the parameter considered in the statistical analysis. There was no significant difference between estrogen and non-estrogen users with respect to years since menopause, DHEAS, estradiol, total or free testosterone, total cholesterol, HDL, LDL, HDL/total Cholesterol ratio, systolic and diastolic blood pressure, smoking and anthropologic measurements.

Table III shows the Pearson correlation coefficients between BMI, waist-to-hip ratio, and Testosterone Ratio Test. As expected, the correlation between BMI and Waist-to-Hip ratio showed strong positive correlation with multiple cardiovascular risk factors. It was surprising, however, that the Testosterone Ratio Test showed as strong a positive correlation with these same factors. None of the independent hormonal parameters, alone, showed as significant correlation.

Table IV showed the Pearson correlation coefficients for the subset using and not using estrogen supplementation.

Discussion

Table V outlines the correlation coefficients in those publications limited to postmenopausal women with covariant analysis of hormonal and cardiovascular risk factors. They reported striking but dissimilar results. This inconsistency may be explained by their omission of the calculated value of Testosterone Ratio Test.

The basis of using this ratio to explain androgenicity in women may be determined by review of the original study of D.C. Anderson (1974). Based on his *in vitro* experiments, the percentage of unbound testosterone increases significantly with dropping SHBG levels. See Figure 5. But, in his experiment he kept the testosterone levels stable. In an *in vivo* study, the amount of unbound or free testosterone is influenced also by the amount of testosterone produced by the individual. Therefore, the expected correlation of the Testosterone Ratio Test to cardiovascular risk factors should be greater than either that of SHBG or free testosterone levels. This was confirmed in our study.

Testosterone binding is of high affinity (K. approximately 10^9 M^{-1}), readily reversible at 37°C and, in men, is nearly saturated since the molar concentration of SHBG binding sites in adult male plasma is only marginally greater than the molar concentration of T. In female plasma the SHBG concentration is twofold higher and the T concentration tenfold lower than in men and therefore most of the binding sites are unoccupied. Estradiol binds less well than Testosterone to SHBG, better than T to albumin, and does not bind significantly to CBG.

Despite the small size of the unbound fraction of steroid hormones, it appears that it is this and not the bound fraction, which is biologically active.

A previous study by Burke and Anderson ((1974) Review Article: Sex Hormone Binding Globulin. *Clin Endocrinology*, 3:69-96) showed that changes in SHBG concentration produce a much greater alteration of percentage unbound T than Estradiol. This is partly because Estradiol binds less well than T to SHBG,

and partly because Estradiol binds better than T to albumin. In the same study it was found that increasing the T concentration over the physiological range did not significantly increase the unbound Estradiol; similarly a marked increase in cortisol did not significantly increase the unbound T by displacement off CBG. [p. 77] (Anderson, 1974).

Zeginiadou et al. (1997) noted that an increase in sex hormone binding globulin "resulted in a rapid transfer of estradiol from albumin to"... "the much more biologically active globulin." Therefore, a drop in sex hormone binding globulin decreases the availability of biologically active estradiol and would be detrimental for the female patient.

<u>Mathematical Basis for **Testosterone Ratio Test**</u>

*Unbound {free} testosterone is proportional to total amount of testosterone.

%U ~ concentration of [total testosterone]

*Unbound {free} testosterone is inversely proportional to SHBG[12]

%U ~ 1/ [SHBG]

Therefore, Unbound Free testosterone %U ~ [total testosterone]/SHBG

%U ~ TRT [Testosterone Ratio Test]

In review of previous authors, strong support was found for Phillips (1977) (free testosterone) and Haffner (1995) (SHBG). It is expected that Hauner's (1994) strong correlation to total testosterone would persist if he had incorporated a Testosterone Ratio Test in his correlation coefficients. Barrett-Connor (1995) and Cauley (1990) may have determined significant correlation if they had

incorporated the Testosterone Ratio Test into their analysis. SHBG was omitted from their published studies referenced. In Table V, Applicant reports free estradiol against total estradiol, sex hormone binding globulin and the Testosterone Ratio Test. The fact that there is no correlation explains why Barrett-Connor (1995) and Cauley (1990) did not correlate with the other researchers.

It is also of interest that estrogen users, noted in Table IV, have lost most of the established risk factors of cardiovascular heart disease. This may imply that: 1) these individuals have a decreased risk of cardiovascular risk factors, (2) estrogen has cardioprotective effects or simply 3) estrogen raises SHBG lowering the TRT.

Although the original premise of androgenicity as a predictor of cardiovascular risk is confirmed by the representation of total testosterone in the calculation of the Testosterone Ratio Test, there may be other factors than testosterone and estrogen that independently relate to SHBG. It is interesting to note that the Testosterone Ratio Test shows an extremely high correlation ($p<.000$) to all the androgens mentioned: DHEAS, Androstenedione, free and total testosterone. Further studies will try to determine what role the non testosterone androgens has on the Testosterone Ratio Test.

Previous information on the relationship of sex hormone binding globulin and disease was confusing. Postmenopausal women have a decreased level of SHBG (Berglund et al., 1996; Bhasin et al, 1996). Yet, decreased SHBG has predicted the development of non-insulin dependent diabetes mellitus in two

populations (Birkeland et al., 1993; Black, 1996). Since both conditions, postmenopausal and insulin resistance exists in our study population, both hypotheses could be applicable. Applicant questions whether it is the underlying change in insulin resistance as seen in the Testosterone Ratio Test that predates the development of both cardiac disease and the diabetic state.

What then comes first, the changes in insulin resistance or the changes in the Testosterone Ratio Test? Haffner stated that insulin excess does not change the sex hormone binding globulin but changes in the sex hormone binding globulin change insulin levels (Bhasin et al., 1996). Pasquali and Nestler disagreed, however, finding that diazoxide administration as an insulin blocking agent resulted in increased SHBG (Faix et al., 1993; Franke and Fuchs, 1955).

A number of reports confirm the inverse relationship between SHBG and insulin. Hyperinsulinemia and insulin resistance have been related to a decreased level of SHBG in postmenopausal women. (Soler et al., 1989; Haffner et al., 1992) Decreased SHBG has predicted the development of non-insulin dependent diabetes mellitus in two populations. (Lindstedt et al., 1990; Haffner et al., 1993) Haffner clearly states that "the alteration in sex hormone, rather than a direct effect of insulin" (1992) causes changes in lipid metabolism. It is our opinion that changes in SHBG influences insulin resistance directly, and thereafter serum levels of insulin.

Conclusion:

The data supports the strongly positive association between the ratio of testosterone and SHBG, the Testosterone Ratio Test (TRT), and recognized cardiovascular risk factors. This association was independent of age and remained significant even after adjustment for the influence of BMI. Applicant's results were consistent with those of previous authors, but more importantly, Applicant's study has added the observation of TRT as an independent cardiovascular risk factor. It was of greater statistical significance than free testosterone, total testosterone or SHBG taken individually. The mathematical calculation of TRT takes into account the total and free testosterone, overall androgenicity, and SHBG. Of particular interest was the observation that in multiple linear regression analysis, TRT was more strongly related to cardiovascular risk factors than Apoprotein B, lipids, blood pressure and insulin levels. In postmenopausal women, increased androgens remain associated with cardiovascular risk factors and this may be best considered by measurement of the Testosterone Ratio Test.

TABLE II: POSTMENOPAUSAL WOMEN: Mean and Standard Deviations

variable	Mean	Standard Dev	Cases
Age	60.6455	11.7675	95
BMI	31.1650	7.0397	95
DM	1.8421	00.3666	95
testosterone	.2422	00.1704	95
TC	223.4947	44.2202	95
HDL	.2513	00.0811	95

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Table III. Summary of the Principle Cardiovascular Risk Factors Pearson Correlation Coefficients ('r' and 'p' values) for entire population

	BMI		WHR		HDL/Chol		TRT	
Factor	r	p	r	p	r	p	r	p
Age	-.206	.009	.065	NS	.032	NS	-.107	NS
Cortisol	-.265	.001	-.020	NS	.064	NS	-.103	NS
androstenedione	.030	N/A	.0176	.026	.001	NS	.401	.000
BMI			.366	.000	-.252	.002	.278	.000
BP diastolic	.240	.005	.207	.012	-.118	NS	.128	.128
DHEAS	.135	.094	.014	NS	-.182	.022	.406	.000
Insulin	.359	.000	.162	.040	.038	NS	.087	.271
HDL/ CHOL	-.252	.002	-.299	.000			-.202	.011
HDL	-.353	.000	-.372	.000	.757	.000	-.196	.014
triglycerides	.194	.016	.149	.057N	-.431	.000	.185	.020
TRT	.401	.000	.211	.007	-.202	0.11		
Testosterone	.101	.213	.076	.335N	-.107	NS	.823	.000
ApoB	.159	.308	.239	.123N	-.702	.000	.128	.421
SHBG	-.564	.000	-	.025	-.291	NS	-.377	.025
WHR	.366	.000			-.299	NS	.211	.007

Table IV. Testosterone Ratio Test Risk Factors
Pearson Correlation Coefficients ('r' and 'p' values)
Total group, Estrogen Users, Non Estrogen Users

	TRT(total=161)		TRT(estrogen=49)		TRT(no=112)	
Factor	r	p	r	p	r	p
Age	-.107	NS	-.285	NS	-.059	NS
Cortisol	-.103	NS	-.257	NS	.060	NS
androstenedione	.401	.000	.297	NS	.485	.000
BMI	.278	.000	.164	NS	.360	.000
BP diastolic	.128	.128NS	.081	NS	.171	.NS
DHEAS	.406	.000	.500	.001	.386	.000
Insulin	.087	.271NS	-.002	NS	.096	.271NS
HDL/ CHOL	-.202	.011	-.291	NS	-.173	NS
HDL	-.196	.014	-.281	NS	-.133	NS
triglycerides	.185	.020	.023	NS	.360	.000
Testosterone	.823	.000	.937	.000	.681	.000
ApoB	.128	.421NS	.128	NS	.182	NS
WHR	.211	.007	.297	.053NS	.184	.053NS
SHBG	-.337	.025	-.337	.025	-.472	.000

TABLE V.

Hormone	Risk Factor	Correlation-Coefficient	Author/year
Free testosterone	Degree of CAD	$p \leq .008$	Phillips, 1997
SHBG	-BMI	$p < .001$	Haffner, 1995
SHBG	-glycosylated HgB	$p < .001$	
SHBG	-diastolic BP	$p < .01$	
SHBG	+HDL & HDL/TC	$p < .001$	
Free Testosterone	+HDL/TC	$p < .05$	
Total Testosterone	+HDL/TC	$p < .01$	
Total Testosterone	+BMI	$p < .01$	
Total Testosterone	+Systolic BP	$p < .01$	
Total Testosterone	+Diastolic BP	$p < .01$	
Estrone	-HDL/TC	$p \leq .05$	
SHBG	+WHR	$p < .001$	Hauner, 1995
Testosterone	+serum insulin	$p \leq .01$	
SHBG	-Triglycerides	$p < .01$	Svendsen, 1993
SHBG	+WHR	$p < .001$	
SHBG	+HDL	$p < .05$	
Androstenedione	-HDL	$p < .05$	
Estradiol	-HDL	$p < .05$	
Estradiol	-TC	$p \leq .01$	
SHBG	+HDL	$p < .001$	Haffner, 1992
SHBG	-Triglycerides	$p < .05$	
SHBG	-serum insulin	$p \leq .001$	
SHBG	+HDL	$p < .07$	Soler, 1989
	-triglycerides	$p < .002$	
Estrone	+triglycerides	$p \leq .003$	

As a result of the Postmenopausal Cardiovascular Study set forth above in Example 6, Applicants determined first by review of significance of various combinations of all hormonal parameters tested that the percentage of unbound testosterone was predictive of both cardiac and diabetic disease states in both Caucasian and African-American women. The Testosterone Ratio Test (TRT) showed as great a predictive value as the Total Cholesterol/HDL-Cholesterol ratio as a risk factor for heart disease, yet the TRT is independent of age which the TC/HDL is not.

Applicant then sought to apply the Testosterone Ratio Tests to adult onset diabetics. The Testosterone Ratio Test appears to offer benefits over other common testing parameters for diabetes as it is stable throughout the day and need not be performed with individual fasting. It should be noted that, in comparison, the standard glucose tolerance test misses more than fifty percent of individuals who have impaired glucose tolerance.

With the significance of the Testosterone Ratio Tests determined in men and women with diabetes, and women with heart disease, an extensive literature search was performed to determine if this same ratio was applicable to other medical conditions. Haffner, Lindstedt, and Hauner confirmed our observation without making reference to the underlying physiology. That is, the Testosterone Ratio Tests correlate directly with insulin levels and insulin resistance.

Normal levels of testosterone for both sexes appear in Table VI. In the United States, total testosterone is measured in ng/dl and sex hormone binding globulin in pmol/L. The conversion from ng/dl to pmol/L is .0347.

Therefore, the TRT is calculated thus: TT x .0347/SHBG.

TABLE VI

Sex	Total Testosterone (TT)	Sex Hormone Binding Globulin (SHBG)	Testosterone Ratio Test (TRT)
Male	>400 ng/dl	<20 pmol/L	0.70 to 1.2
Female	<40 ng/dl	>40 pmol/L	0.01 to.035

In a study of twenty adult onset diabetic men on dialysis, the TRT was found to be 0.2 in eight of the ten men. The other two men were newly diagnosed and had levels at 0.5.

In a study of thirty-five men and women with confirmed myocardial disease, all the men had TRT levels below 0.4.

The Testosterone Ratio Test and its components provide a screening tool for the recognition and treatment of insulin resistance, Syndrome X and for the application of testosterone replacement.

In two men with cluster headaches and a low TRT, testosterone supplementation to therapeutic levels at the normal male maximum resulted in the complete absence of cluster headaches/migraines for two years.

In normal men, a total testosterone concentration is greater than 400 mg/dl. Sex hormone binding globulin less than 20, thus presenting a testosterone ratio normally between approximately 0.7 to 1.2.

For insulin resistant diabetic men, the testosterone ratio is generally 0.05 and lower.

For normal women, the concentration total testosterone is generally less than 30 mg/dl. The concentration of sex hormone binding globulin is generally greater than 40, and the ratio of the two ranges is normally between 0.01 to 0.04.

For insulin resistant diabetic women, the ratio is generally 0.06 and greater.

The effective pharmacological doses of parenteral testosterone and/or its derivatives which are effective as anti-insulin resistant agents in both men and women range from approximately 200 mg monthly to 1200 mg. More preferably, the effective pharmacological dose ranges from measurement of serum testosterone is 1000 ng/dol to 3000 ng/dl.

The preferred methodology for achieving a pharmacologically therapeutic range can be achieved by placing 200 to 900 mg of testosterone pellets subcutaneously in the abdominal fat or hip fat pads every one to two months. The total dosage of testosterone pellets and the frequency may be increased until the testosterone therapeutic range is reached, there are clinical effects of testosterone on insulin resistance, and the TRT is in a therapeutic range with the sex hormone binding globulin reduced or stable. The goal of repeated measurement of the

Testosterone Ratio Test is to achieve a normal or supranormal male ratio in both men and women. For diabetic women do not experience improved glycemic control from the increase in sex hormone binding globulin that results from exogenous estrogen.

The advantages of testosterone therapy include:

(1) testosterone, when used in pharmacological doses described above, is applicable for cases of insulin resistance;

(2) testosterone enhances the action of both insulin and oral hypoglycemic agents;

(3) testosterone does not induce any drug reactions or side-effects when combined with either insulin or hypoglycemic agents;

(4) testosterone does not induce insulin coma or hypoglycemia at any dosage; and

(5) testosterone lowers serum cholesterol, triglycerides and increases high-density lipoproteins in the therapeutic range noted above.

Once the therapeutic range noted above has been confirmed, the goal for the patient remains a euglycemic state with minimal utilization of insulin or oral hypoglycemic agents.

In view of the teaching presented herein, other modifications and variations of the present inventions will be readily apparent to those of skill in the art. The foregoing drawings, discussion, and description are illustrative of some embodiments of the present invention, but are not meant to be limitations on the

practice thereof. It is the following claims, including all equivalents, which define the scope of the invention.

Any patents or publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. These patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The present examples along with the methods, procedures, treatments, molecules, and specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope of the claims.

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